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Synthesis of fructo-oligosaccharides using grape must and sucrose as raw materials

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ABSTRACT

Grape must market has been rising and there is an increasing interest to use it as a "natural" replacement for traditional sugars. Food or beverages with prebiotic compounds, including fructo-oligosaccharides (FOS), emerge as an alternative for the new health style trend.

The aim of this work was to investigate whether the combination of grape must with sucrose was a suitable raw material for the synthesis of FOS. This way, a prebiotic syrup containing fructose and FOS, potentially useful for the formulation of foods and beverages, could be obtained. The main process consisted of three stages, namely conditioning of grape must (oxidation of the initial glucose concentration, stage 1), synthesis of FOS [incorporation of 20, 30 and 55% (*w*/w) sucrose, and 3.5% *v*/v Viscozyme L – 4.2 U/mg-, stage 2], and conditioning of the final product (oxidation of the glucose generated during the synthesis, stage 3).

At stage 1, glucose concentration decreased from 222.8 mg/mL to 47.2 mg/mL, representing a decay of about 80% regarding the initial concentration of glucose. At stage 2, incorporating 20% (*w*/w) sucrose was not enough to impulse FOS synthesis. In turn, although 30 and 55% (*w*/w) sucrose produced very similar concentrations of total FOS (DP3 + DP4), 55% (*w*/w) sucrose led to higher glucose generation and less DP4 formation. Hence, 30% (w/w) sucrose was the condition selected for the synthesis and further conditioning of the obtained product (stage 3). In these conditions, the final product consisted of more than 30% of short chain FOS (19% and 13% of DP3 and DP4, respectively), 55% fructose and less than 11% of glucose and sucrose.

Considering that fructose has approximately double sweetening power than glucose, the obtained syrup has a bigger sweetening power in comparison with the original grape must, also providing the prebiotic benefits of FOS.

1. Introduction

Wine production and consumption is concentrated in different regions throughout the world namely Europe (Spain, Italy, France, Portugal), America (Argentina, Chile, USA) and also South Africa and Australia, all competing for a share of above 25 billion liters world market (Mateo & Maicas, 2015; Zacharof, 2017). Grape must is obtained from early steps in winemaking, accounting about 80 L per 100 kg of grapes (Melamane, Strong, & Burgess, 2005; Moletta, 2007; Musee, Lorenzen, & Aldrich, 2006). Different legislations stimulate producers to leave a defined percentage of must as such to avoid overproduction of wine, and consequently, a decrease in the price (Marshall, Akoorie, Hamann, & Sinha, 2010). Therefore, must is usually marketed as concentrated syrup and due to its high content of glucose and fructose (1:1 ratio), its main application is as a "natural" replacement for traditional sugars (high fructose corn syrup and refined sucrose) (Granato, Carrapeiro, Fogliano, & van Ruth, 2016). For this reason, grape must market has been rising, in order to supply an ingredient to formulate other food products including juices, soft drinks, syrups, baby foods, pharmaceuticals and sweets, among others (Coelho et al., 2018; Eyduran, Akin, Ercisli, Eyduran, & Maghradze, 2015).

One of the most important trends in food consumption has been the demand for healthy food or ingredients to enrich traditional processed foods or beverages (Bigliardi & Galati, 2013). In this sense, functional foods with addition of prebiotic compounds [substances selectively used by host microorganisms conferring a health benefit (Gibson et al., 2017)] emerge as an alternative for this health style trend (Granato, Branco, Nazzaro, Cruz, & Faria, 2010; Martins et al., 2013). Among them, fructo-oligosaccharides (FOS) have a great economic importance as they are extensively used in the formulation of functional foods and infant formula, with about 60% of the sweetening power of sucrose

Abbreviations: FOS, Fructo-oligosaccharides; *DP*, *Degree of polymerization*; $C_{FOS[t]}$, *FOS concentration at t = t (mg/mL)*; C_{FOS0}, *FOS initial concentration (mg/mL)*; $C_{g[t]}$, *Glucose concentration at t = t (mg/mL)*; C_{g0}, *Glucose initial concentration (mg/mL)*; *DP3*, *1-Kestose*; *DP4*, *Nystose*; *DP5*, *1F-fructofuranosylnystose*; ε, *Absolute relative error (%)*; *FOS*, *Fructooligosaccharides*; k_{FOS}, *FOS logistic kinetic constant (1/hour)*; k_g, *Glucose logistic kinetic constant (1/hour)*; *t*, *Reaction time (hour)*; t_{FOSm}, *FOS characteristic kinetic time (hour)*; t_{gm}, *Glucose characteristic kinetic time (hour)*; V_{exp}, *Experimental Value*; V_{pred}, *Predicted Value*.

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(Romano, Santos, Mobili, Vega, & Gómez-Zavaglia, 2016; Romano, Schebor, Mobili, & Gómez-Zavaglia, 2016).

From a chemical approach, FOS are short chain oligosaccharides composed of fructose units linked by β-(2→1) glycosidic bonds, and a single D-glucosyl unit at the non-reducing end. Most of them are composed of mixtures of oligosaccharides with degrees of polymerization (DP) between 3 and 10 (Campbell et al., 1997). At an industrial level, FOS can be obtained by enzymatic synthesis (Chacon-Villalobos, 2006) using sucrose as substrate and fructosyltransferases (β-fructofuranosidase, EC 3.2.1.26 or β-D-fructosyltransferase, EC 2.4.1.9) as biocatalysts (Beine, Moraru, & Nimtz, 2008; Ghazi et al., 2007; Vega & Zuñiga-Hansen, 2011, 2012, 2014). Transfructosylation reactions involve the cleavage of the β-2,1-glycosidic bond and the transfer of fructosyl moieties from carbohydrates acting as donors onto any acceptor other than water, such as other sugars (among them fructose) with different specificities (Fujtta, Hara, Hitoshi, & Kitahata, 1990; Vega & Zuñiga-Hansen, 2014). In general, most fructosyltransferases have also a hydrolytic activity, so that the synthesis of FOS involves the hydrolysis of sucrose and different reactions of synthesis and hydrolysis occurring simultaneously both in parallel and in series (Vega & Zuñiga-Hansen, 2014). The final products of such reactions are mixtures of FOS with different degrees of polymerization (DP), together with fructose and glucose (Romano, Santos, et al., 2016; Romano, Schebor, et al., 2016). This latter product can be removed using chromatographic methods or glucose-oxidase treatments, thus increasing the yield of FOS (Romano, Schebor, et al., 2016; Vega & Zuñiga-Hansen, 2014).

Considering that grape must is a largely available industrial by-product, majorly composed of glucose and fructose, the aim of this work was to develop a protocol to obtain FOS using grape must and sucrose as reactants. This strategy promotes an innovative method for adding value to grape must, by using it in combination with sucrose for the synthesis of FOS. Such strategy would enable the obtaining of a prebiotic syrup enlarging the market of grape must as a functional ingredient for the formulation of foods and beverages.

2. Materials and methods

2.1. Materials

Grape must was kindly donated by Kineta S.A. (Mendoza, Argentina). Enzymes: Viscozyme L (Novozyme, Denmark) (56 FU/mL; FU: fructosyltransferase units) and glucose–oxidase–peroxidase enzymatic kit were obtained from Nutring S.A. (Buenos Aires, Argentina). 1-Kestose (DP3), nystose (DP4) and 1^F-fructofuranosylnystose (DP5) standards were purchased from Wako Chemicals (Richmond, VA, USA). Sucrose and calcium hydroxide were obtained from Sigma Chemical (St. Louis, MO, USA). Activated charcoal was supplied by Cicarelli (Santa Fe, Argentina) in a granular form, with 1.5 mm mean particle diameter. Ethanol was obtained from Anedra (Buenos Aires, Argentina).

2.2. Methods

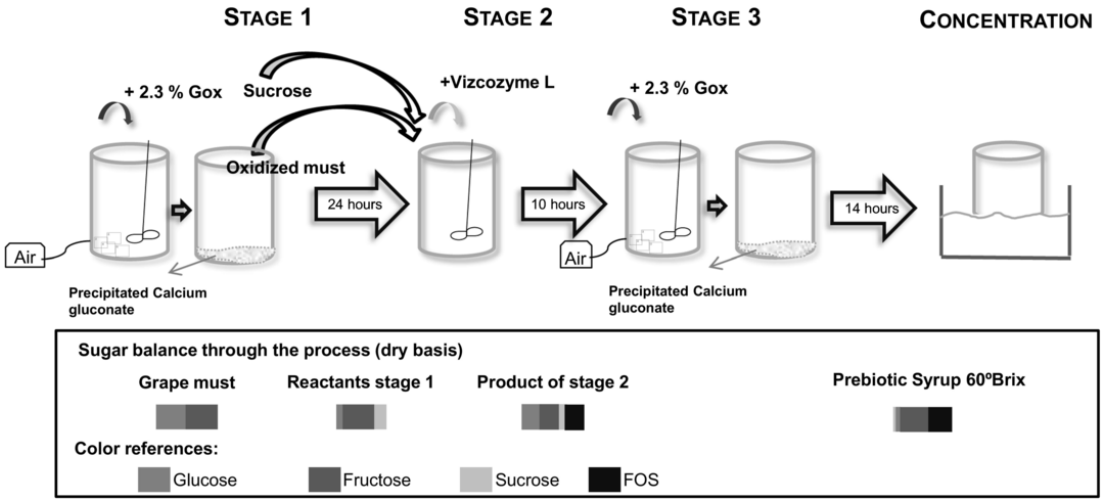
2.2.1. Conditioning of grape must and synthesis of FOS

Grape must was characterized by determining its composition (HPLC, Section 2.2.2), pH (pHmeter Altronix, EZDO-PC) and °Brix (Refractometer, Hanna Instruments). The proposed method for obtaining FOS was composed of three stages: conditioning the grape must (stage 1), synthesis of FOS (stage 2), and oxidation of the glucose remaining in the reaction medium (stage 3). Scheme 1 shows the experimental protocol for FOS synthesis.

2.2.1.1. Conditioning grape must (stage 1) Must was diluted in distilled water (1:1) to decrease the viscosity of the medium. The volume of reaction (600 mL) was constantly stirred at 100 rpm and aerated at a flow rate of 5 L/min. The reaction was performed at pH 4.5 and 25 °C. To control foam generation, 500 μL of silicone food grade anti-foam (AE TN 22, Argentina) was added. Oxidation was performed during 24 hoursh by adding 2.3% (*v*/v) glucose-oxidase (Nutring S.A., Decazyme, 1500 IU/L) as biocatalyst. A 0.25 M solution of $Ca(OH)_2$ was continuously pumped with a peristaltic pump (Gilson Miniplus 3, USA- at 0.2 mL/min) to counteract the generation of gluconic acid (precipitation as calcium gluconate). The enzyme was inactivated by heating the reaction medium at 100 °C for 5 minutesmin.

2.2.1.2. Synthesis of FOS (stage 2) Different combinations of sucrose and grape must were investigated for the synthesis of FOS. They consisted on solutions formulated with oxidized must (obtained in stage 1) and sucrose at different concentrations (20% w/w, 30% w/w and 55% w/w, relative to total carbohydrates in the mixture). The composition of the three reactants used for the synthesis is presented in Table 1. Viscozyme L (3.5% v/v, 56 FU/mL; FU: fructosyltransferase units) was used as biocatalyst. The synthesis was performed for 6 h at 50 ± 1 °C with stirring (100 rpm). The reaction progress was followed by taking samples at regular intervals (every 1 h, up to a total of 6 h for all the condition evaluated). The reactions were stopped by inactivating the enzyme at 100 °C for 5 min.

2.2.1.3. Oxidation of glucose from the reaction product (stage 3) Glucose remaining as secondary product of the enzymatic reaction was oxidized as explained in 2.2.1.1 for must. After inactivation, the enzymes were removed from the reaction volume, by cooling at 4 °C for 12 hoursh and centrifugation at 10000 × *g* (Beckman, USA) during 15 min-



Scheme 1. Experimental design scheme of all the stages involved in the process.

Table 1
Composition of the different reactants used for the synthesis of FOS.

	20% w/w	30% w/w	55% w/w
Sucrose % w/w	20	30	55
Glucose % w/w	14	12	8
Fructose % w/w	66	58	37

In all cases, substrates were formulated in distilled water. The final concentration of the three solutions was 40 *w/v* %.

utesmin. The supernatants, containing FOS syrup, were stored for further concentration by heating samples in a thermostatic bath at 85 °C for 6 hours up to a final concentration of 65°Brix.

Reactions corresponding to stages 1, 2 and 3 were all performed in duplicate.

2.2.2. HPLC analysis

The composition of carbohydrates in must (Section 2.2.1), throughout the syntheses and after oxidation of glucose was determined by HPLC in a Perkin-Elmer Series 200 equipment (Massachusetts, USA) with refractive index detector and autosampler. The chromatographic column used was Sugar Pak I column (10 μm, 6.5 × 300 mm) with Guard Pak LC pre-column inserts (10 μm) (Waters, Milford, MA, USA). Column and detector temperatures were maintained at 80 °C. Samples were diluted in accordance with the detection range of HPLC equipment (1–1.5 mg/mL), filtered through 0.22 μm Millipore Durapore membranes (Billerica, MA, USA) to preclude any contaminant particle and eluted with milli-Q water (mobile phase) at a flow-rate of 0.5 mL/min. Chromatograms were integrated using WinPCcrom XY, versión 2.0 (Eng. Santiago Sobral, Buenos Aires, Argentina). Standards of fructose, glucose, sucrose, 1-kestose (DP3), nystose (DP4) and 1F-fructofuranosyl nystose (DP5) were used to determine their retention times and check the linear range of the measurements. The composition of samples was determined by assuming that the area of each peak was proportional to the weight percentage of the respective sugar of the total sugar mass (Boon, Janssen, & van der Padt, 1999) and the accuracy of such an assumption was checked by making a material balance. All compositions were analyzed in duplicate.

2.2.3. Statistical analysis and validation of mathematical models

The experimental data were subjected to the analysis of variance. Comparison of means was conducted by using Fisher's least significant difference test, with a 5% significance level. Kinetic mathematical models were analyzed with Origin Pro 8.5 and Matlab 7.8.0 fitting tools. Predicted values, V_{pred}, were compared with experimental data, V_{exp}, and the absolute relative error, ε %, between them was estimated.

$$\varepsilon\ (\%) = \frac{1}{n}\sum_{i=1}^{n}\left(\frac{\left|V_{pred} - V_{exp}\right|}{V_{pred}}\right)100 \quad (1)$$

3. Results and discussion

3.1. Stage 1

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The oxidation of glucose from must was the first step for the obtaining of FOS, keeping in mind that grape must composition consists mainly in glucose and fructose (47% and 50% *w*/w, respectively, relative sugar composition measured as described in Section 2.2.2). Given that free glucose in the reaction mixture for FOS production acts as an inhibitor for the fructosyl-transferring reaction (Vega & Zuñiga-Hansen, 2014), there is a need to lower its concentration. Hence, the first stage of this process was to oxidize glucose. It is worth mentioning that even though the optimal conditions for glucose-oxidase activity are pH [5–6] and [30–40]°C (Biyela, du Toit, Divol, Malherbe, & van Rensburg, 2009) the reaction was performed at pH 4.5 and 25 °C, in order to counteract the interaction O_2:SO_2 (Danilewicz & Standing, 2018). Grape must often contains traces of SO_2 to preserve it from aerial oxidation. In this sense, acid pH maintains O_2:SO_2 interaction less efficient and lower temperature favors O_2 solubility in the reaction mixture. Fig. 1 shows glucose concentration decay during stage 1. Glucose concentration decrease can be characterized by three phases: up to 3 hourh, it decreased at a very slow rate, after this and till 10 hourh of reaction it decreased faster, and then till the end the decay rate remained constant. Pickering, Heatherbell, and Barnes (1998), studied the optimization of glucose conversion to reduce alcohol wine using similar pH and temperature conditions and reported an exponential decrease of glucose concentration. Although their results are apparently more promising, the concentration of glucose in the initial substrate (Riesling and Müller-Thurgau juice) is about half of that present in the grape must used in this work (85.6 mg/mL *vs* 222.8 mg/mL), the percentage decay of glucose being similar in both works (*ca.* 80%). Moreover, Pickering et al. (1998) used higher amount of enzyme per mL of grape must and the enzyme specific activity was higher than the one used in the present work. It is worth mentioning that at industrial scale one of the most important factors that affect production costs is the enzyme price (Martins Meyer, Melim Miguel, Rodríguez Fernández, & Dellamora Ortiz, 2015). In this sense, Biyela et al. (2009), who also studied enzymatic reduction of glucose content of grape juice to obtain reduced alcohol wine, reinforce the idea that higher amounts of enzyme improve glucose reduction yield. Therefore, a compromise between enzymatic costs and products yield must be considered when defining the conditions for glucose oxidation.

Different fitting equations were tested to describe the kinetic behavior of glucose concentration decrease (zero order, first order, Weibull, dose-response and logistic). A logistic mathematical model (Eq. (2)) turned out the best one to describe the experimental curve ($R^2 > 0.98$):

$$C_{g[t]} = \frac{C_{g0}}{1 + e^{\left(-kg\left(t - t_{gm}\right)\right)}} \quad (2)$$

being t reaction time (hour), C_{g0}, $C_{g[t]}$ glucose concentration (mg/mL) at $t = 0$ and at $t = t$, respectively, t_{gm} the time at which $C_{g[t]}$ is the average between C_{g0} and C_{gf} (glucose concentration at the end of the reaction), and kg (1/hour) a logistic kinetic constant. The predicted values for these parameters are shown in Table 2. The absolute relative er-

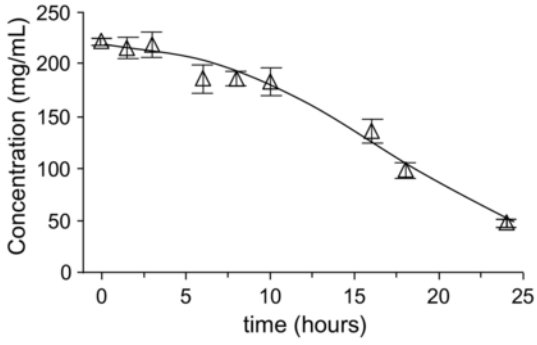

Figure 1. Glucose concentration decay during stage 1. Experimental measurement (glucose, triangles) and logistic model (full line; Eq. (2)).

Table 2
Fitting parameters of the logistic model and its standard deviation.

	C_{g0} (mg/mL)	t_{gm} (hour)	k_g (1/hour)	C_{FOS0} (mg/mL)	t_{FOSm} (hour)	k_{FOS} (1/hour)
Value	228.7	17.0	−0.18	112.6	2.6	0.91
SD	10.5	0.8	0.03	8.2	0.34	0.020

ror calculated from Eq. (1) was 4.3% confirming a good precision of the kinetic model proposed.

3.2. Stage 2: FOS synthesis

Sucrose is generally used as substrate for the synthesis of FOS and the reaction is carried out *via* the breaking of the β-(2–1)-glycosidic bond and the transfer of the fructosyl moiety onto any acceptor other than water, such as sucrose or a fructo-oligosaccharide (Flores-Maltos et al., 2016). Even when the specificity of fructose as fructosyl acceptor is lower than that of sucrose (Fujtta et al., 1990), the possibility of using fructose from grape must as an acceptor was evaluated with the aim of investigating a novel application to add it value. Taking this into account, the strategy in this work consisted in adding the oxidized grape must obtained in stage 1 to different concentrations of sucrose in order to evaluate their effect on the products' composition (Table 1). Figure 2 shows the relative composition after 6 hoursh of synthesis under the reaction conditions reported in Section 2.2.1.2. Sucrose was the only component that did not show significant differences when using the three different reactant combinations ($p > 0.05$). There can be seen that adding oxidized must to a 20% (*w*/w) sucrose solution was not enough to impulse the synthesis of FOS, as FOS (DP3 + DP4) only represented 4% of the composition of products (Figure 2).

When comparing the other two conditions (30 and 55% (w/w) sucrose), the total FOS composition in the mixture after six hours of synthesis was very similar (21.8% and 21.0% for 30% and 55% of sucrose, respectively) (Figure 2). Even though, these two conditions produced similar amounts of DP3 (18.2 ± 1.7% *vs* 15.0 ± 2.5%), 55% (w/w) of sucrose entailed an increase in glucose relative composition (37.0% *vs* 23.4%). On the other hand, when the reactants contained 30% (w/w) of sucrose, a higher amount of DP4 was obtained (Figure 2). These results suggest that, 30% of sucrose turned to be the most promising concentration to leverage the use of grape must keeping sucrose as a minor component. For this reason, further experiments in this work were carried out using such relative concentration of reactants.

As the product composition after 6 hoursh of synthesis, only includes a low amount of glucose (well-known enzymatic inhibitor) (Alvarado-Huallanco & Maugeri Filho, 2011; Lorenzoni, Aydos, Klein, Rodrigues, & Hertz, 2014; Romano, Santos, et al., 2016), it was decided to continue the reaction process.

Figure 3A shows the evolution of product relative composition throughout 10 hoursh of synthesis. Sucrose decreased along the whole process, from 32 ± 2.6% to 10.3 ± 2.3% (squares in Figure 3A). In turn, fructose concentration decreased constantly the first five hours of reaction from 57.6 ± 3.9% to 39.3 ± 3.5% and then remained constant, representing 42.2 ± 2.9% of the total carbohydrates in the reaction medium (empty circles). Regarding FOS production, DP3 and DP4 were obtained at different concentrations. DP3 appeared at the beginning increasing up its composition till five hours of reaction (full black diamonds). Afterwards, it remained quite constant with a slight decrease at the end of stage 2 (between 7.5 and 10 hoursh). Despite the particular behavior regarding the production of DP3 and DP4, total amount of FOS increased constantly the first 5 hoursh and then, slowed down the rate of increase reaching a plateau between 7 and 10 hoursh (full gray diamonds in Figure 3A and Figure 3B). Lorenzoni et al. (2014) who studied FOS synthesis using the same enzyme (Viscozyme L), reported a similar behavior. On the other hand, Vega and Zuñiga-Hansen (2011) also used fructosyltransferases (Rohapect CM) to determine the best conditions for producing short chain FOS from sucrose obtaining a high percentage of 1-kestose. In the mentioned work, authors reported similar evolution of FOS composition, although they also used higher enzyme activity and substrate concentration (72% (*w*/*v*) sucrose). As our goal was to minimize the use of sucrose and make maximum usage of grape must syrup, the possibility of using a crude commercial enzyme in small quantities makes it more rentable when thinking in pilot scale production.

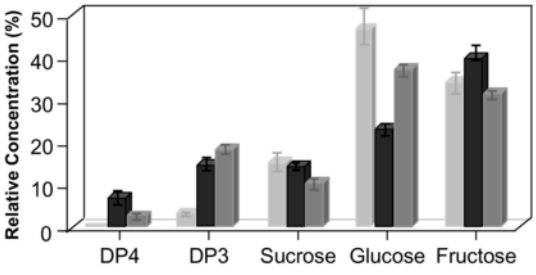

Figure 2. Relative composition (%) of the products obtained after 6 hoursh of synthesis, using different concentrations of sucrose as co-substrate. Light gray bars, 20% (*w*/w) sucrose, black bars, 30% (w/w) sucrose, dark gray bars, 55% (w/w) sucrose.

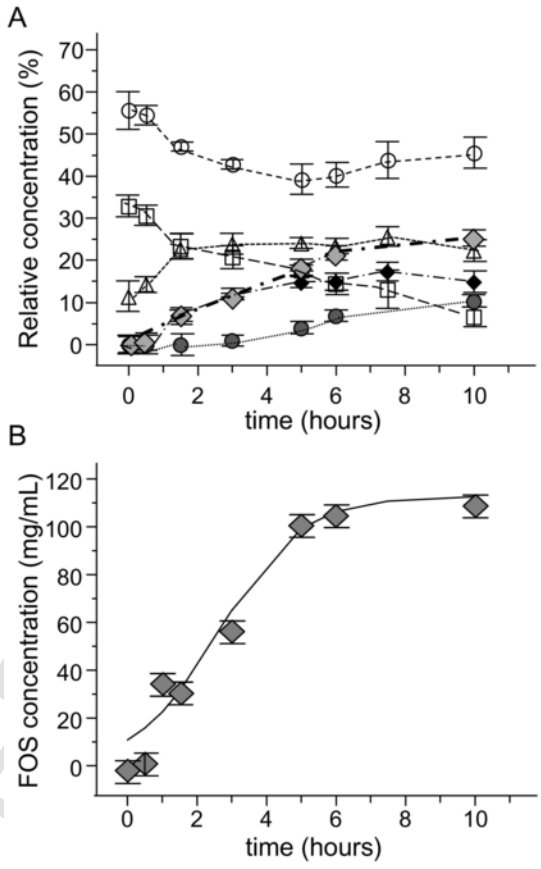

Figure 3. A. Relative composition of the reaction medium during stage 2. Substrate: oxidized must and 30% w/w sucrose. Squares, sucrose; triangle, glucose; empty circles, fructose; full black diamonds, DP3; full gray circles, DP4; full gray diamonds, total FOS (DP3 + DP4). For a better visualization of reactions, symbols were connected with different types of lines. B. Evolution of total FOS concentration during stage 2. Full gray diamonds, total FOS (DP3 + DP4); full line, logistic model (Eq. (3)).

From a mathematical point of view, a kinetic logistic model (Eq. (3), $R^2 > 0.96$) was also the one which better described the behavior of FOS production (DP3 + DP4) (Figure 3B, full line) (absolute relative error ε: 10%).

$$C_{FOS[t]} = \frac{C_{FOS\,0}}{1 + e^{(-k_{FOS}(t - t_{FOSm}))}} \quad (3)$$

being t reaction time (hour); C_{FOS0}, $C_{FOS[t]}$ (mg/mL) FOS concentration at $t = 0$ and at $t = t$, respectively; t_{FOSm} the time (hour) at which $C_{FOS[t]}$ is the average between C_{FOS0} and C_{FOSf} (mg/mL, FOS concentration at the end of the reaction); and k_{FOS} a logistic kinetic constant

(1/hour). The predicted values for these parameters are depicted in Table 2.

With the aim of analyzing FOS yield rate, first derivate of Eq. (3) was calculated, as shows Eq. (4):

$$\frac{dC_{FOS\,[t]}}{dt} = \frac{C_{FOS\,0}\, e^{(-k_{FOS}(t-t_{FOSm}))}}{\frac{1}{k_{FOS}}\left(1 + e^{(-k_{FOS}(t-t_{FOSm}))}\right)^2} \quad (4)$$

From Eq. (4) maximum FOS rate production was 23.2 (mg mL/hour) and it was reached at $t = 3$ hoursh coinciding with the maximum relative concentration of glucose (24%). Afterwards it decreased till a minimum value of 0.13 (mg mL/hour), while glucose composition remained constant at 24%, confirming the inhibitory effect of glucose concentration on the production of FOS. Alvarado-Huallanco and Maugeri Filho (2011) described a kinetic mathematical model for the synthesis of FOS using partially purified enzyme, with sucrose as substrate. This model consists of an equation for predicting rate of concentration variation of each reagent and product involved in the reaction. Regarding the maximum rate of production of DP3, which almost coincides with the maximum production of total FOS (Romano, Sciammaro, Mobili, Puppo, & Gómez-Zavaglia, 2018), authors reported 48.0 mg mL/hour (estimated value from equation 7 in Alvarado-Huallanco & Maugeri Filho, 2011), achieved at much larger times of reaction (20 hoursh, with partially purified enzyme, and 50% sucrose as substrate). Although the yield of FOS in the present work was lower, considering that our scope was to enlarge the uses of grape must, the products obtained after synthesis provide a syrup that can be catalogued as a nutritional valuable sweetener enriched with prebiotics.

3.3. Stage 3. Obtaining the final product

Glucose oxidation reaction was performed and, as expected, there was found a very similar behavior that the one described in Section 3.1, with the exception that at this stage the initial concentration of glucose was approximately half the one reported for grape must (216.0 ± 0.6 *vs* 98.3 ± 2.1 mg/mL, respectively). Figure 4 shows initial and final carbohydrates relative composition of stage 3. Glucose composition was reduced from 22.6% to 3.7%. Of course this caused an increase in the relative contribution of the rest of the components. The relative carbohydrate composition of the final syrup is 4% of glucose, 7% of sucrose, 57% of fructose and 32% of FOS. Thus, a high concentrated fructose syrup with more than 30% of short chain FOS, (19% and 13% of DP3 and DP4, respectively) and less than 11% glucose and sucrose was obtained. Then, water was removed (water content slowed down from 60 ± 0.6% to 34 ± 0.8), to obtain a concentrated syrup of 65 ± 0.1°Brix with the same composition of carbohydrates. It must be considered that the European Commission recommended dose for infant formulas regarding GOS and FOS must not exceed 0.8 g/100 mL (of a combination of 90% GOS and 10% FOS)

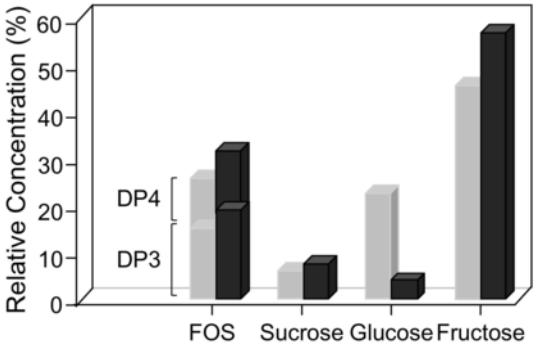

Figure 4. Relative composition of carbohydrates before (gray bars) and after (black bars) stage 3.

(Braegger et al., 2011). Considering that the obtained syrup provides near 0.2 g FOS/mL, this means that only 0.4 mL are needed to cover the recommended FOS dose (0.08 g/mL) for infant formulas.

Taking into account the main objective of this study, this final carbohydrate composition is quite promising. On the one side, high fructose content enhances the sweetening power in comparison with the original grape must, given that fructose has approximately double sweetening power than glucose. Moreover, this syrup as a sweetener product provides all the benefits of FOS, being excellent replacers for mono and disaccharides in food products without affecting the palatability, flavor, body, and mouthfeel, the opposite, it enhances all this quality attributes (Moser & Wouters, 2014). From a nutritional point of view, FOS are assumed to be vegetal fibers for being non-digestible polysaccharides, so they improve and optimize the nutritional composition of many foods: "sugar out, fiber in" (Moser & Wouters, 2014). In addition, as it was previously mentioned FOS consumption entails health benefits stimulating the growth and activity of beneficial bacteria in the gut, thus promoting a good balance of intestinal microflora and decrease gastrointestinal infections (Arrizón et al., 2014; Gibson, Rastall, & Fuller, 2003).

4. Conclusions

Grape must is a by-product available in high amounts as regulations force producers to leave certain percentages as such, to avoid overproduction of wine. Therefore, must is majorly used as sweetener in the beverages industry. Taking into account that the world sweetener global market is valued close to US$ 12 Bn and will reach a market valuation of about US$ 14 Bn by 2027 (futuremarketinsights.com), combining grape must with sucrose for the synthesis of FOS represents an innovative approach to add it value. The final product was a syrup rich in FOS (DP3 and DP4) and fructose and with low concentration of glucose and sucrose. Considering the prebiotic properties of FOS and the sweetening power of fructose the obtained product appears as a promising one. It represents an adequate functional ingredient to sweeten different beverages, without interfering with their quality attributes (palatability, flavor, body, and mouthfeel), improving the nutritional composition and entailing gastrointestinal health benefits.

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Competing interests

The authors declare that they have no competing interests.

Author's contributions

M.U. did experimental work, analyzed results and wrote the manuscript; N.R did experimental work and discussed results, E.K. discussed results, A.G.-Z coordinated the work (analysis of results, discussion and minor writing of the manuscript). All authors have approved the final version of the manuscript.

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